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## FURTHER STUDY OF SELF-ASSEMBLY IN T2 PHAGE TAIL SHEATHS

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We showed in earlier works [1, 2] that brief exposure to a 2 M KOH solution results in T2 phage tail sheaths with a sedimentation constant of about 107S breaking down into smaller fragments with constants of the order of 10 - 30S. Subsequent dialysis is followed by protein reaggregation and appearance of structures resembling the original tail sheaths. This finding was the first indication that T2 phage tail sheaths can be reconstructed under artificial conditions. However, it was still unclear why the process of self-assembly ceases with the formation of structures as long as the intact sheaths and goes no further. It would be logical to assume that under *in vitro* conditions, when there is no specific regulation of the process, self-assembly may continue and give rise to longer structures, as in the case of reaggregation of BTM protein [3]. Moreover, we observed [4], as did Kellenberger and Boy de la Tour [5], that lysates obtained after the escape of T2 and T4 phages from cells may contain structures, the so-called polysheaths, whose length far exceeds that of normal sheaths.

Thus, our purpose in this work was to make a more detailed study of the processes of disaggregation and self-assembly in T2 phage tail sheaths and to determine the conditions under which protein reaggregation is not sharply limited.

БиоСТАМ

УГРОДИАЛУЧСКОЙ

### Method

The method of preparing highly purified T2 bacteriophage and isolating the tail sheaths has already been described [6].

The sedimentation characteristics were determined in a Hitachi analytic ultracentrifuge. The relative viscosity of the solutions of the sheaths was measured at 24° in a protein concentration of 6.25 - 7.0 mg/ml in a Ostwald viscosimeter. The results were expressed in units of the reduced viscosity.

An 11B Hitachi microscope was used with electron-optic magnification of 50,000 and voltage of 75 kv. The preparations for the electron-microscopic study were contrasted with a 2% uranyl acetate solution and covered by a Formvar film.

The turbidity of the protein substances was determined in an SF-4A spectrophotometer at 500 m $\mu$ .

### Results

The tail sheaths were isolated into a separate fraction by means of alkali treatment and differential centrifugation. The high degree of purity of the preparation was confirmed by sedimentation and electron-microscopic studies. The sedimentation coefficient of the whole tail sheaths under the given conditions (solvent - water) was about 110S. Electron microscopy showed that the preparation was a homogeneous suspension of contracted tail sheaths (Fig. 1). Analysis of the electron-microscopic photographs confirmed that the mean diameter of the sheaths was 250 Å while the length was 350 Å. The number of constituent protein subunits ranged from 132-144.

1000Å

Fig. 1. Electron-microscopic photographs of the original T2 phage tail sheath

In the present work, as in the earlier one, we treated the tail sheaths with KOH in order to dissociate them into subunits. In doing so we made a detailed study of the effect of various KOH concentrations on some physicochemical properties of the sheaths.

Since one of the most effective methods of studying the degradation of protein structures is sedimentation analysis, we investigated the effect of various concentrations of the alkali on the sedimentation coefficient of these structures in the ultracentrifuge. We found a distinct relationship between the value of the sedimentation coefficient of the tail sheaths and the amount of alkali added. Figure 2 shows that in solutions containing the alkali in low concentrations (up to 0.01 M, which corresponds to a pH of 10.7), the sedimentation coefficient does not change, i.e., the structure of the sheath remains intact. And only a further increase in the concentration of the alkali to 2 M caused the sedimentation coefficient to decrease gradually from 110S to 1.5 - 2.0S (cf. table).

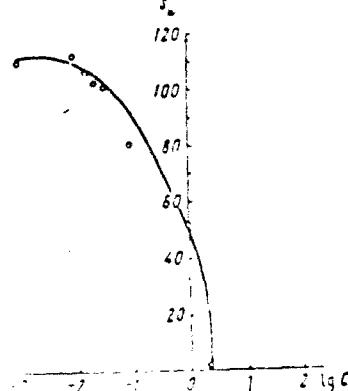


Fig. 2. Relationship between the sedimentation coefficient of T2 phage tail sheaths and KOH concentration

Water solutions of the tail sheaths with a protein concentration of  $3.0 \pm 0.3$  mg/ml were used. C - alkali concentration

The changes were more complex in a 1M KOH solution. Brief exposure to the alkali (10-15 min) reduced the sedimentation coefficient to 52S, but this decrease was reversible. With a longer exposure (30-60 min) the coefficient decreased to 1.5-2.0S and subsequent dialysis produced marked aggregation, resulting in the formation of heavier and more heterogeneous material. A high concentration of the alkali (2 M) was much more effective, causing a rapid (within 15 min) dissociation to subunits (1.5 - 2.0S). Subsequent

**Sedimentation Coefficients of T2 Phage Tail Sheaths With Different KOH Concentrations**

KOH concn. MOL/L	pH	Concen. mg/ml	$s_{20}^0$	KOH concn. MOL/L	pH	Concen. mg/ml	$s_{20}^0$
0	7.0	3.0	110.87	0.040	11.75	2.85	101.82
0.010	10.7	2.85	112.10	0.1	12.7	3.3	79.53
0.018	11.0	3.3	106.09	1.0	—	3.0	52.0
0.025	11.3	3.3	102.84	2.0	—	3.0	1.5-2.0

1 - KOH concentration, M

2 - Protein concentration, mg/ml

dialysis, as in the case of the 1 M solution, intensified the formation of heavy and heterogeneous aggregates.

The sedimentation coefficients of these aggregates were considerably higher than those of the original whole tail sheaths. The results of the experiments suggested that in KOH solutions of low concentration (0.018-0.1 M) or after brief exposure to 1 M KOH solution, the protein helix from which the tail sheath is constructed stretches like a spring without dissociating into subunits. In doing so the sedimentation coefficient decreases because of the more asymmetrical structure. Removal of the alkali causes the helix to contract again. In more concentrated solutions of the alkali (2 M) or after longer exposure to a 1 M KOH solution, the helix not only stretches, but it appears to dissociate into subunits with a sedimentation constant of 1.5 - 2.0S. In this event subsequent dialysis necessarily leads to the formation of longer units.

The results of experiments with determining the viscosity of a solution of tail sheaths upon the addition of increasing amounts of KOH confirmed the correctness of our assumption on the dissociation process. We found that an increase in the concentration of the alkali increased the viscosity of the solution, the maximum values occurring with the 1 M KOH solution (Fig. 3). Adding more alkali to the solution sharply decreased its viscosity. These findings are consistent with the results of sedimentation analysis and show that in solutions with an alkali concentration of up to 1M, where there are reversible changes in the sedimentation coefficient, the tail sheaths become more asymmetrical, i.e., they stretch. Higher concentrations of the

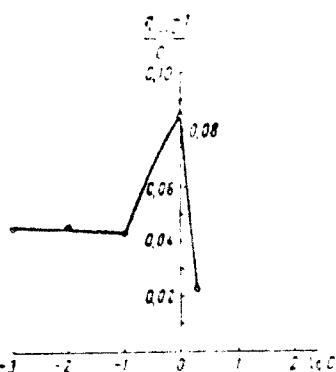


Fig. 3. Relationship between the reduced viscosity of T2 phage tail sheaths and KOH concentration

Water solutions of the tail sheaths with a protein concentration of 0.625% were used. The measurements were made at 24° immediately after the alkali was added. C' and C - protein and alkali concentrations, respectively

alkali (above 1 M), when the sedimentation coefficient decreases sharply to 1.5 - 2.0S, cause the sheaths to dissociate into sub-units, as reflected in decreased viscosity of the solution. Thus, an alkali concentration of about 1 M is a threshold concentration, but, as our experiments showed, the dissociation of the tail sheaths into subunits necessarily goes through the stage of stretching of the protein helix. It is quite evident from Fig. 4 that the viscosity of the suspension increases upon the addition of the alkali, but starts to decrease immediately thereafter. This complex set of changes in the quaternary structure of the sheaths had to be taken into account in all the control determinations.

These results would have been incomplete without an electron-microscopic study of the process of degradation and aggregation of the tail sheath protein. The high degree of resolution of the electron microscope enabled us to analyze carefully the structure of the tail sheaths before and after they were treated with the alkali.

In the presence of 1 M KOH, the preparations were found to contain instead of the normal sheaths a large number of threads (Fig. 5, a) forming a mat-like structure. When the alkali concentration was increased, the threads disappeared and the entire visual

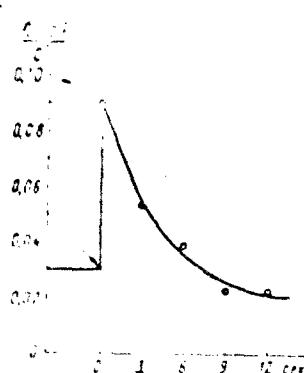


Fig. 4. Change in the reduced viscosity of T2 phage tail sheaths in a 1 M KOH solution

Water solutions of the tail sheaths with a protein concentration of 0.625% were used. The measurements were made at 24°. The arrow indicates the time when the KOH solution was added.

C' - protein concentration

field was studded with subunits (Fig. 5, b). Removal of the alkali by dialysis altered the picture considerably. The preparations treated with weak alkali (0.1 M) contained instead of threads structures that were outwardly indistinguishable from the original tail sheaths (Fig. 5, c), i.e., the helix apparently contracted. But in cases where 1 M and sometimes 2 M alkali was used, dialysis resulted in the appearance of a large number of tubules tending to vigorous side-to-side aggregation (Fig. 5, d, e). Measurement of the smallest diameter of the tubules showed that it matched the diameter of the intact tail sheath (about 250 Å). They were usually longer than the original sheaths. In many cases units were formed in which it was difficult to distinguish the individual tubules owing to the strong interaction of the tubules with one another. The packing of the subunits in the tubular structures could be discerned very infrequently and then only with great difficulty. We believe that these structures were polysheaths formed *in vitro* by the self-assembly of the sheath protein. The dialyzed preparations obtained after treatment with 1 M alkali contained occasional threads (Fig. 5, f) from which the polysheaths were apparently constructed following further aggregation. Also, a few normal sheaths about 350 Å long were invariably present in the preparations. This showed that part of the original protein helices merely stretched but did not dissociate

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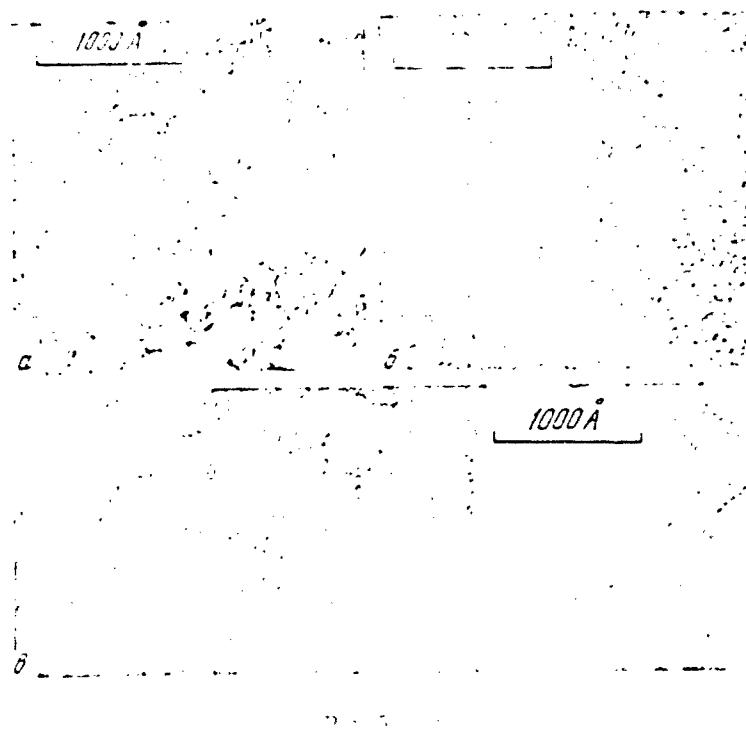


Fig. 5, a-c. Electron-microscopic photographs of the degradation and reaggregation products of T2 phage tail sheaths.

a - threads of stretched tail sheaths in 1 M KOH;  
b - protein subunits obtained after dissociation of the tail sheaths in 2 M KOH; c - dialyzed preparation of tail sheaths after treatment with 0. 1 M KOH; d - e - polysheaths formed by artificial self-assembly of protein subunits (preparation dialyzed after treatment with 1 M KOH for 30 min); f - initial stage of reaggregation of sheath protein after treatment with 1 M KOH for 30 min.

Fig. 5, d - f. Found on following page.

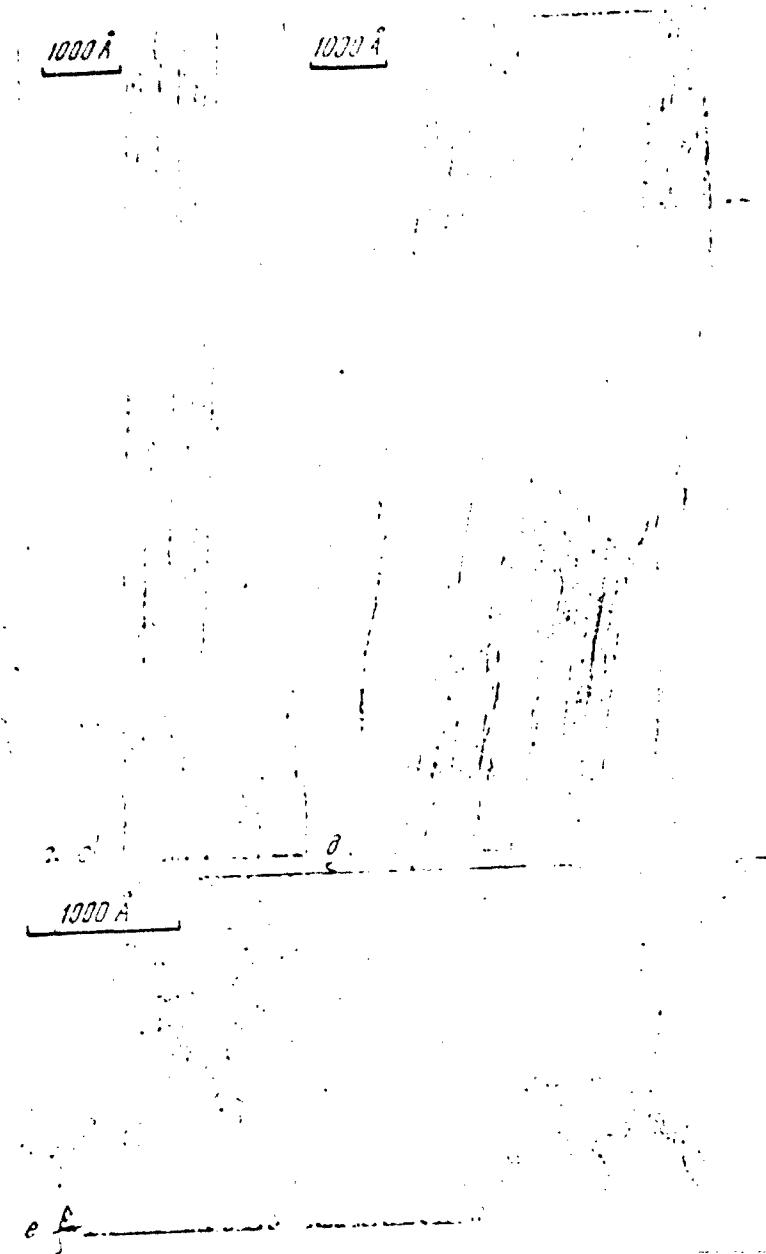


Fig. 5, d - f.

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into subunits. It will be noted that self-assembly of the subunits into polysheaths occurred only after a comparatively brief exposure to the alkali (usually no more than an hour). A longer exposure disrupted the orderliness of the process and instead of polysheaths amorphous units appeared.

#### Discussion

Sarkar et al. [7] showed that tail sheaths are stretched slightly by high concentrations of ATP. In our experiments the use of strong treatment made it possible to go further and induce more severe structural impairment, although in both cases it would appear that the quaternary structure of the sheath was stretched as a result of electrostatic repulsion.

The polysheaths formed by self-assembly had the structure of contracted sheaths, indicating that the process of contraction resulted from deep irreversible changes in the protein molecules and subsequent reorganization of the entire structure. We cannot say for sure that the alkali was responsible for the sheaths stretching into structures corresponding to the stretched sheaths in the intact phage. On the other hand, it is reasonable to believe that the artificial stretching of the sheaths reflected a change in the already reorganized structure. The possible ways of reorganization of the sheaths after contraction were recently discussed in the literature [8, 9], although many aspects of the problem remain obscure.

We showed in our earlier studies [1, 2] that strong exposure to alkali destroys the tail sheaths while markedly decreasing the sedimentation coefficient. However, we were unable to observe the formation of polysheaths, apparently due to the insufficient resolving power of the electron microscope that we used, especially since the polysheaths exhibited an extremely powerful tendency to side-to-side aggregation, thus making it difficult to distinguish the individual tubules. However, we were able to induce a restoration of the original tail sheaths and the somewhat shorter cylinders. This restoration was apparently caused by contraction of the stretched protein strands and by aggregation of a few subunits. Now that we have obtained data on the self-assembly of polysheaths, we can say in full confidence that this process takes place during the formation of tail sheaths *in vitro*, although the manner of self-assembly of the stretched tail sheath is still unknown.

### Conclusion

Sedimentation analysis, viscosity determinations, and electron microscopy showed that treatment of T2 phage tail sheaths with KOH solutions in concentrations of 0.018-0.1 M and brief exposure to 1 M KOH results in stretching of the protein helix which constitutes the cylinder of the tail sheath. This stretching is reversible and it is followed by a decrease in the sedimentation coefficient from 110S to 52S. Increasing the alkali concentration to 2 M and longer exposure to 1 M KOH causes the stretched protein helices to dissociate into subunits with a sedimentation coefficient of about 1.5 - 2.0S. Subsequent dialysis results in self-assembly of the subunits which ends in the formation of polysheaths.

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